

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

**Characterization of the Mechanistic Differences in Splicing of SF2/ASF
RS Domain-Independent and RS Domain-Dependent Pre-mRNAs**

By

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SR proteins are essential pre-mRNA splicing factors that bind to pre-mRNA via their RRM domains and are thought to recruit components of the splicing machinery through protein-protein interactions mediated by their RS domains. Our lab previously discovered that splicing can occur for some but not all pre-mRNAs *in vitro* with a mutant SR protein SF2/ASF lacking its RS domain (" Δ RS") as a sole source of SR protein. Thus pre-mRNAs could be classified as either RS domain-dependent or RS domain-independent based on their ability to be spliced with an SR protein lacking its RS domain.

To identify pre-mRNA sequence elements that confer the RS domain requirement for splicing of RS domain-dependent pre-mRNAs, we tested in the *in vitro* splicing assay with SF2/ASF and Δ RS a large number of pre-mRNAs in which specific sequences were mutated or replaced by sequences from RS domain-independent pre-mRNAs. We have demonstrated that improvement of the pyrimidine tract, an essential intronic splicing signal upstream of the 3' splice site, abrogates the RS domain requirement. We have also identified sequence elements spanning the 5' splice site which confer RS domain-dependence. Collectively these findings suggest that RS domain-dependence is caused by a defect in intron definition, the assembly of spliceosomal complexes across the intron to establish the locations of the 5' and 3' splice sites prerequisite to splicing catalysis.

To try to understand how Δ RS can support splicing *in vitro* for some pre-mRNAs, we tested the abilities of a series of mutant SF2/ASF proteins to splice various RS domain-dependent and RS domain-independent pre-mRNAs. Deletion of the short N-terminal segment preceding the first RRM domain increased the amount of splicing supported by both SF2/ASF and Δ RS. Surprisingly, deletion of this inhibitory segment in the context of Δ RS results in a protein which supports splicing for both RS domain-independent and RS domain-dependent pre-mRNAs, indicating that the RS domain is dispensable for splicing *in vitro*. However, characterization of splicing of RS domain-dependent pre-mRNAs with the Δ N Δ RS protein reveals a kinetic dependence on the RS domain such that RS domain-dependent pre-mRNAs are spliced more slowly with Δ N Δ RS than with SF2/ASF or Δ NSF2/ASF.

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